ELSEVIER

Biochimica et Biophysica Acta 1587 (2002) 1–6

BIOCHIMICA ET BIOPHYSICA ACTA

BBA

www.bba-direct.com

Review

Gene repair and mutagenesis mediated by chimeric RNA–DNA oligonucleotides: chimeraplasty for gene therapy and conversion of single nucleotide polymorphisms (SNPs)

Ian R. Graham*, George Dickson

Centre for Biomedical Science, School of Biological Sciences, Royal Holloway-University of London, Egham TW20 0EX, UK

Received 5 September 2001; received in revised form 24 January 2002; accepted 4 February 2002

Abstract

Gene augmentation is an attractive and viable approach in treatment of inherited diseases, despite its limitations, such as the eliciting of host immune response, and the sustainability of gene expression. Therefore, alternative therapeutic approaches are being investigated, such as the use of chimeric RNA–DNA oligonucleotides (chimeraplasts), in which a mutated allele that already exists in an affected individual can be corrected. Although the only gene defects that can be corrected by chimeraplasty are point mutations, and the correction frequencies are variable, it has been observed that intracellular delivery of oligonucleotides is likely to be more efficient than that of plasmid DNA or viral vectors. Furthermore, corrected genes are expressed from their autologous promoters, thus ensuring correct spatial and temporal expression. Here we report on the recent progress made in the field of chimeraplasty, and the problems encountered. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Chimeric oligonucleotide; Gene correction; Gene targeting; Gene therapy; Mismatch repair; Point mutation

1. Introduction

The conventional approach to the treatment of inherited disease by gene therapy is that of gene augmentation. That is, the supply, in *trans*, of a functional copy of the gene which is defective in the diseased state. This is mostly achieved by the introduction of functional cDNAs or, if the gene is short enough, genomic DNA sequences, into plasmid or viral vectors. The viruses that are in widespread use in gene delivery research include adeno-associated viruses (AAV), retroviruses (RV), lentiviruses such as HIV, SIV and EIAV, and most commonly, adenoviruses (Ad). Each virus family has distinct advantages over others, but they also have their drawbacks. For example, Ad has the ability to infect quiescent, or nondividing cells. This makes it a far more attractive tool for engineering skeletal muscle cells than RV, for example, which can only infect dividing cells. However, RV have the advantage over Ad of being able to insert the therapeutic transgene into the host genome, which often results in more sustainable levels of expression.

Due to their often pathogenic nature, it is important that the viral vectors are used simply as delivery vehicles, and that they only perform one transduction event. This is achieved by deleting large amounts of the viral genome, ensuring that the subsequent viruses are "replication defective". This has the added advantage of creating more space in the genome to insert foreign (i.e. therapeutic) DNA. A major drawback of many viruses is the limited amount of nucleic acid that can be successfully packaged into infectious virus particles, so large cDNAs, for example dystrophin, cannot be inserted in their complete state, and efforts have to be made to establish which, if any, regions of the coding sequence may be deleted while still generating a functional protein.

Even when a complete gene delivery system has been generated, problems are encountered when its therapeutic benefit is assessed in vivo. Most viruses, even though replication defective, will still elicit a host immune response, whether cell-mediated or humoral. Furthermore, in cases of inherited disease in which a protein is completely absent in the diseased state, the introduction of a therapeutic copy of that gene is itself likely to induce an immune response, which is clearly undesirable, and will result in no therapeutic benefit whatsoever. If the chosen viral vector does not

* Corresponding author. Tel.: +44-1784-443870; fax: +44-1784-434326.

E-mail address: i.graham@rhul.ac.uk (I.R. Graham).

PII: S0925-4439(02)00068-6

integrate its genome into that of the host, there is likely to be a severely reduced longevity of expression, especially in rapidly dividing cells, but if integration does occur, there are serious risks associated with insertional mutagenic effects on the host genome. Moreover, if all of these problems can be overcome, there is the potential for heterologous promoters to be down-regulated within a short space of time, leading to a loss of therapeutic gene expression.

Not all of the above problems are insurmountable, and the use of gene augmentation remains an attractive and viable option as a potential therapy for inherited disease. However, the search for alternative approaches to genetic medicine has continued, and within the past 5 years, a new technique has been developed that has the potential to overcome the majority of the problems associated with gene augmentation.

Chimeric RNA–DNA oligonucleotides (now termed chimeraplasts) that are directed to a particular sequence in a gene have been shown to be able to bring about site-specific point mutations within that sequence (Table 1). If such a point mutation had the effect of reversing a mutation that causes a genetic disease, this method of gene correction could be used as a genetic medicine, and would have a number of distinct advantages over gene therapy as achieved by gene augmentation.

For example, there is no restriction to the size of the gene that can be corrected. So in cases of Duchenne Muscular Dystrophy, there is the clear potential for point mutations that disrupt the reading frame of the dystrophin gene to be corrected, whereas delivery of the complete cDNA cannot easily be achieved using viral vectors. Moreover, there is no risk of the generation of replication-competent pathogens, as is potentially the case when engineering virus backbones. The risks associated with insertional mutagenesis by certain viral vectors that integrate at random sites in the genome are also overcome completely. Perhaps the most important of all the advantages that chimeraplast-mediated gene correction has over gene augmentation is that the corrected gene is expressed using its autologous promoter, to an appropriate level in a spatially and temporally relevant manner. The principal barrier to the efficacy of the chimeraplasty method is that the only defects that can be corrected are substitutions and single base insertions or deletions.

The use of chimeraplasts to induce and correct point mutations was first described in 1996 by Eric Kmiec and coworkers, then at Thomas Jefferson University in Philadelphia, PA, USA [1]. In this landmark study, chimeraplasts were used to correct a nonfunctional allele of a human alkaline phosphatase gene on an expression plasmid in Chinese hamster ovary (CHO) cells. The rate of correction was demonstrated to be in the order of 30% to 40%, by histochemical staining for the presence of functional alkaline phosphatase, by an allele-specific hybridisation technique, and by direct sequencing of polymerase chain reaction (PCR) products.

The first demonstration of the technique in a genomic context also came from Kmiec's lab [2]. Chimeraplasts were designed to correct the sickle cell anemia mutation (β^S) in β-globin to the wild-type (β^A) allele. When introduced to lymphoblastoid cell lines that were homozygous for the β^S allele, correction to β^A was observed at a detectable level, as adjudged by genomic Southern hybridisation, PCR-RFLP and direct sequencing of PCR products. Importantly, this was achieved in the absence of any significant increase in random mutations, demonstrating the safety of the technique as a potential genetic medicine. Shortly afterwards, the correction of an alkaline phosphatase gene in a genomic context was performed, with an intracellular correction efficiency in excess of 40% [3].

The next significant advancement in the method came the following year, with the first demonstration of the success of chimeraplasty in a whole animal. Chimeraplasts designed to induce a Hemophilia B-causing mutation into the clotting factor IX gene were introduced into rat models by tail–vein injection. Conversion of the wild-type to the mutant allele was observed at levels of up to 40%, following administration of two doses of chimeraplast. A single dose using less oligonucleotide yielded a correction frequency of about 11% [4].

Table 1
Examples of the widespread use and potential of chimeraplasty

Gene	Syndrome	System used	Conversion rate quoted (%)	Chimeraplast design[a]	Reference
Alkaline phosphatase		CHO cells	30	68mer (10–5–10)	[1]
β^s globin	Sickle cell anaemia	Lymphoblastoid cells		68mer (10–5–10)	[2]
Alkaline phosphatase		Hepatoma cells	11	68mer (10–5–10)	[3]
Factor IX	Haemophilia B	Rat, in vivo	40	68mer (10–5–10)	[4]
Kanamycin-resistance		Cell-free extract	0.1	68mer (10–5–10)	[14]
UGT1A1	Crigler–Najjar syndrome	Gunn rat, in vivo	20	76mer (10–9–10)	[5]
Acetolactate synthase	Herbicide resistance	Tobacco		68mer (10–5–10)	[10]
		Maize		68mer (10–5–10)	[11]
Tyrosinase	Albinism	Mouse, in vivo		68mer (10–5–10)	[6]
Dystrophin	DMD	*mdx* mouse, in vivo	20	78mer (12–6–12)	[7]
		GRMD dog, in vivo		74mer (10–5–13)	[8]
Apolipoprotein E	Atherosclerosis	CHO cells	35	68mer (10–5–10)	[20]

[a] The total length of the oligonucleotide and the composition, in numbers of nucleotides, of the chimeric strand (RNA–DNA–RNA).

It is evident from this and other studies that the liver is particularly amenable to the uptake of chimeraplasts [4,5], but other organs, including skin [6] and muscle [7,8], are also well suited as targets for the technique, and can result in potentially therapeutic levels of correction, approaching 20% in the dystrophin-deficient *mdx* mouse [7]. Liver targetting has been further enhanced by the use of galactosylated delivery agents such as PEI, which bind to the asialoglycoprotein receptor on the surface of hepatocytes [9].

The technique has also been demonstrated in plants, showing the potential to introduce herbicide resistance into tobacco [10] and maize [11,12], by modification of the acetolactate synthase (acetohydroxyacid synthase) gene. While this correction has been observed only at very low levels, the application of chimeraplasty to genetic modification in this way may overcome many of the problems associated with the introduction of exogenous genes into random sites in the plant genome. As a result, the technique may become of enormous agronomic benefit.

The methods used in the analysis of chimeraplast-mediated correction have rightly been the focus of much scrutiny, to ascertain that the results that have been quoted have not been biased in favour of higher apparent correction frequencies. For example, an early criticism of PCR-based evidence was that a long-lived chimeraplast might itself act as a primer and, as it contains the ‘‘corrected’’ sequence, skew the result towards high correction efficiency [13]. However, this has been discounted by corroborative PCR-independent

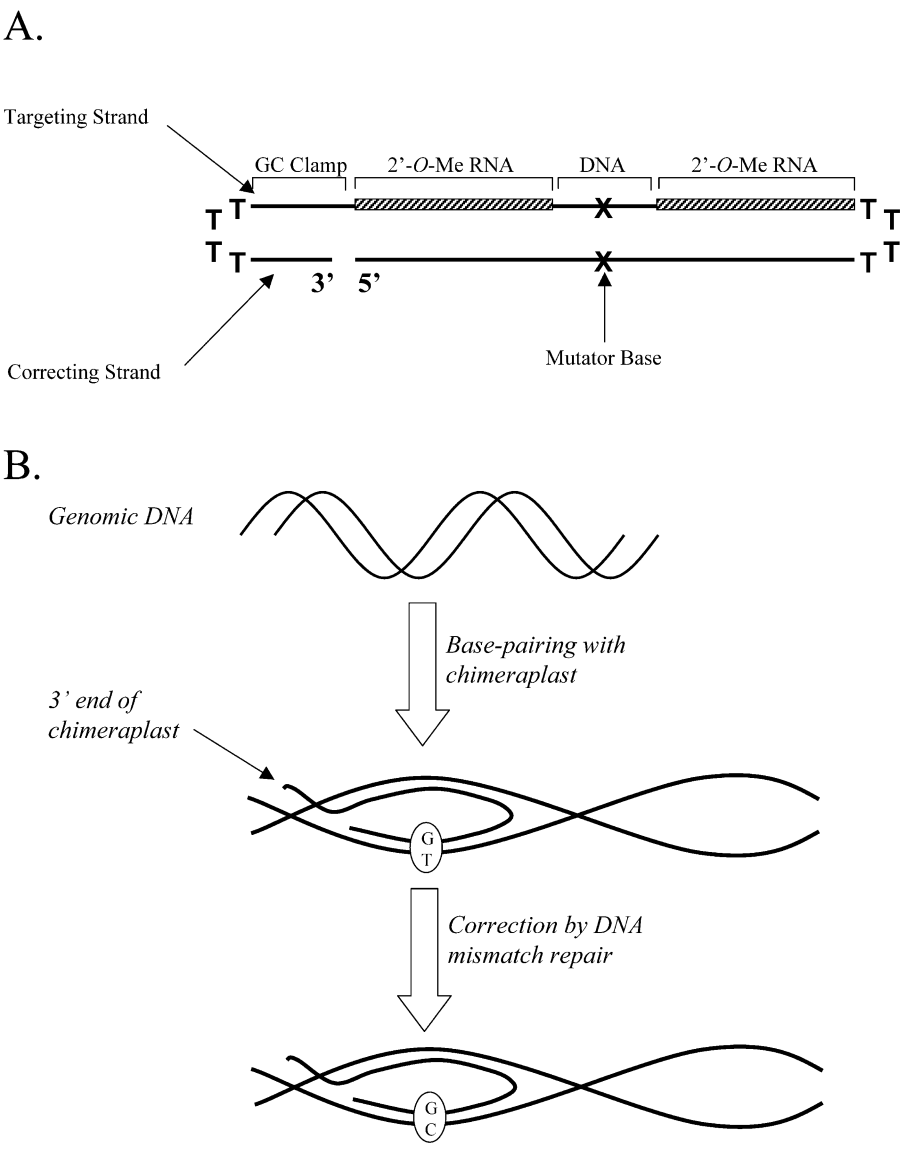

Fig. 1. (A) The structure of a typical chimeraplast, in which the chimeric (targeting) strand is entirely base-paired to the all-DNA (correcting) strand. (B) Schematic representation of chimeraplast action. Strand invasion by the chimeraplast is initiated by RecA, and stabilised by base pairing of the targeting strand to the upper strand of the genomic DNA. The base pairing of the correcting strand to the lower strand of the genomic DNA creates a mismatch, which is repaired. This example depicts a T to C mutation.

techniques, including Southern blots of genomic DNA, protein analyses and functional biochemical assays. Moreover, the emergence of real-time PCR as a routinely available technique will also counteract the aforementioned argument.

2. Chimeraplast design

The design of the chimeraplast used in the first experiments [1] has remained largely unchanged to the present day. It consists of 68 nucleotides (nt) that base-pair to give a double-stranded molecule, in which one strand is comprised entirely of DNA, and the other strand comprises of two runs of 10 nt of 2′ -*O*-methyl (2′ -*O*-Me) RNA, separated by a 5 nt stretch of DNA (Fig. 1A). A short region predicted to have a high melting temperature (a GC clamp) is located at the 3′ end, and four T residues separate the double-stranded regions, allowing the energetically favourable formation of tight hairpins. The base that is to be mutated lies at the centre of the stretch of five DNA nucleotides on the chimeric strand, and has its complement at the centre of the all-DNA strand. This ensures that the chimeraplast is capable of intramolecular base pairing along its entire length.

More recently, attempts have been made to study empirically the structure and efficiency of action of a chimeraplast [14,15]. A large number of molecules were tested, all of which were designed to correct an inactivating mutant in a bacterial kanamycin-resistance gene in a cell-free extract. Following incubation with the chimeraplast, the plasmids were transformed into *Escherichia coli* cells. In this way, an accurate estimate of the efficiency of the correction was determined by an analysis of the number of kanamycin-resistant colonies. The observations reported in these studies suggest that changes can be made to the first generation chimeraplast in order to improve its performance. For example, if the targeting strand is made up entirely of 2′ -*O*-Me RNA, rather than being interspersed with a stretch of DNA, the efficiency is increased by about 40%. This enhancement in activity may be improved further by having a mismatch between the genome and only the correcting (all DNA) strand. That is, the 2′ -*O*-Me RNA strand is entirely complementary to the genome, and there is a mismatch between the two strands of the chimeraplast. While this would reduce the intramolecular melting temperature, the melting temperature of the targeting strand to the genome would be increased.

3. Mechanism of action

The improvements made to the structure described above have implications for the mechanism of chimeraplast action (Fig. 1B). It is presumed that invasion of the host genome is initiated and stabilised by the chimeric strand; the T_m of a hybrid between DNA and 2'-*O*-Me RNA is higher than that of either DNA–DNA or DNA–RNA, and will thus be more stable. Indeed, if the chimeric strand is replaced by one that is comprised entirely of DNA, the ability of the oligonucleotide to induce gene correction is reduced by a factor of 2, compared to the "classical" design [15]. Following base pairing of the chimeric strand to the genomic target, the DNA strand is then able to base-pair with the complementary strand. Whether this occurs solely by Watson–Crick base pairing, or involves an element of Hoogsteen base pairing is not clear. If Watson–Crick base pairs are used exclusively, there is clearly no prospect for the chimeraplast to bind both intra- and intermolecularly. Following the invasion of the genome by the chimeraplast, the point mutation in the genomic DNA that is mismatched with the central base on the all DNA strand of the chimeraplast is corrected. It was originally thought that both strands of a first-generation chimeraplast could induce mutations, but analyses of structural variants have shown this not to be the case. Indeed, if only the chimeric strand is mismatched with the genomic DNA, the conversion frequency is reduced by 95%, compared to a chimeraplast that has mismatches on both strands.

It is not altogether clear as to the mechanism by which the invasion and correction occurs, but recent evidence suggests that the initial invasion is mediated by RecA and its mammalian homologues. RecA is unable to catalyse the invasion of duplex DNA by single-stranded oligonucleotides, but if the complement of the oligonucleotide is also included in the reaction, a displacement loop (D-loop) is formed that is stable following the dissociation of RecA [16]. It is proposed that chimeraplasts are able to form a "complement-stabilised" D-loop, and that the mismatch that exists between the chimeraplast and the genome, along with the recognition of the unusual four-stranded structure, leads to repair of the region [15]. The actual mechanism of mismatch repair is not well understood, but presumably proceeds via excision of a region of the genome in the vicinity of the mismatch and the D-loop, followed by DNA replication by DNA polymerase δ, using the all-DNA strand of the chimeraplast as a template. The process is dependent on a number of proteins, including MSH2, and is much more efficient than homologous recombination. The dependence of MSH2 in chimeraplasty has been demonstrated in cell-free extracts, but the role of DNA polymerase δ remains unclear due to the lack of effect of its inhibitor aphidicolin [14], and the repair may have been completed following introduction of the treated plasmid into *E. coli*.

It is possible that the model systems that have been used to assess the efficiency and mechanism of chimeraplast activity are dependent on plasmid (i.e. supercoiled) DNA for activity, and that the mechanism by which gene correction by chimeraplasts occurs in the mammalian genome is somewhat different. Whether or not that is true remains to be seen, but there is clear evidence from an increasing number of studies that chimeraplasts can be effective in mammalian cell cultures and whole animal models.

Recent studies have also examined the mechanism by which a single-stranded oligonucleotide might achieve gene correction. It is apparent, from the use of model systems such as those described above to test chimeraplasts, that single-stranded oligonucleotides that correspond to the all DNA (correcting) strand of a chimeraplast are able to achieve conversion frequencies similar to the intact chimeraplast [17,18]. Conversely, oligonucleotides conforming only to the chimeric (targeting) strand are incapable of directing correction. The mechanism of strand invasion, the first step of DNA repair systems, is likely to be different, as is the intermediate structure in the process, given that RecA cannot catalyse the invasion of a single-stranded oligonucleotide into duplex DNA unless this occurs at one end of the duplex, or if the duplex is in the form of supercoiled DNA. Furthermore, extracts made from eukaryotic cells with mutations in the MSH pathway have demonstrated the lack of dependence on MSH2 or MSH3 for correction by single-stranded oligonucleotides, and presumably the whole mismatch pathway, in stark contrast to the action of chimeraplasts [15,17]. However, the advent of single-stranded oligonucleotides as alternatives to the traditional chimeraplast is a very important advance in the field of targeted gene correction. A major contributor to the variability of success with chimeraplasts has been the difficulty in synthesis and purification of the reagents (see below). These relatively short DNA oligonucleotides containing fewer modifications are much easier to synthesise and purify in large quantities than chimeraplasts, and are considerably less costly, so are likely to be more attractive should the technique become approved for clinical use.

Recent criticism has been levelled at the chimeraplast technique in three areas [19]. First, that the technique is somewhat variable. Reported correction efficiencies have varied from 0.1% in cell-free extracts to as much as 40% in whole animal models of human disease. It is not clear at this stage what are the factors that contribute to this variability, but one inconsistency between groups could be the quality of the chimeraplast reagents themselves. However, work in our laboratory has shown both very high efficiency, in the case of the conversion of apolipoprotein E single nucleotide polymorphisms (SNPs) in CHO cells [20], and in the case of the introduction of certain atheroprotective genotypes of Apolipoprotein AI, while others appear more refractory [21]. All of the reagents used in these studies were manufactured by the same supplier (MWG Biotech, Ebersberg, Germany), and while they appear to be of equivalent purity and integrity, suggesting that there are some sites that are more amenable to correction than others, it is possible that minor differences between batches of chimeraplast could contribute to this variation, and needs to be addressed further. It might be proposed that GC content (which would have an influence on the stability of chimeraplast-genome hybrids) is key. This may be the case, since work in our laboratory has shown high levels of correction in contexts that have a high GC content [20,21], but a direct correlation has yet to be established. There is also an apparent hierarchy of the correction efficiencies achieved with different mismatches, with, for example, G to C changes being more efficient than A to T [15].

Second, the likelihood of undesired mutations being generated by the nonspecific interaction of the chimeraplast with the genome has not been addressed in great detail. A sequence of 25 nucleotides would be expected to occur at random every 10^{15} base pairs, or approximately once in 375,000 human genomes. Thus, a standard chimeraplast with a 25 base pair homology to the genome would be expected to interact only with the specified sequence. Clearly, if the region of homology is reduced substantially, the probability of nonspecific effects will be higher. Two pieces of work have given some indication of the potential risk involved. Chimeraplasts that were designed to test the role of the chimeric (RNA–DNA) strand, and which are able to direct only very low levels of correction of a kanamycin-resistance gene, were shown to introduce non-complementary mutations into the gene, but at the correct site [15]. Moreover, sequencing of an entire 10 kb plasmid containing the targeted kanamycin-resistance gene, following chimeraplast treatment, has demonstrated that the only mutation was at the correct position (M. Blaese, personal communication).

The third area of criticism is concerned with the low number of published studies that have originated from independent laboratories [19]. While it is true that the majority of studies have come from the laboratories of Kmiec and Steer, it is evident that there is increasing success by other groups. For example, the work on ApoE [20] and ApoAI [21] SNPs has been performed entirely independently of those two laboratories, and we would endorse the suggestion that a network be set up [19,21], allowing the frank and free exchange of results and concerns.

4. Conclusions

It is evident that the use of chimeraplasts and single-stranded oligonucleotides to achieve genetic correction at pathologically significant loci is still a technique of enormous promise, and one which will be of benefit in cases of disease that cannot be effectively managed by conventional gene augmentation therapy. The advances made in the past year in the understanding of the mechanisms and pathways by which correction is brought about will increase our knowledge of the method, which will inevitably lead to overcoming the problems that have become apparent in the 5 years since its inception. The question of whether it will become a widespread method of combating inherited disease remains unanswered. There are clearly organs, such as the liver, which are more amenable to its use than others. This may limit the applicability of the technique, but as the controversial issues surrounding it are discussed openly, the mythology will inevitably be replaced by facts that will

allow chimeraplasts and single-stranded oligonucleotides to reach their true potential.

References

[1] K. Yoon, A. Cole-Strauss, E.B. Kmiec, Targeted gene correction of episomal DNA in mammalian cells mediated by a chimeric RNA. DNA oligonucleotide, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 2071–2076.

[2] A. Cole-Strauss, K. Yoon, Y. Xiang, B.C. Byrne, M.C. Rice, J. Gryn, W.K. Holloman, E.B. Kmiec, Correction of the mutation responsible for sickle cell anemia by an RNA–DNA oligonucleotide, Science 273 (1996) 1386–1389.

[3] B.T. Kren, A. Cole-Strauss, E.B. Kmiec, C.J. Steer, Targeted nucleotide exchange in the alkaline phosphatase gene of HuH-7 cells mediated by a chimeric RNA/DNA oligonucleotide, Hepatology 25 (1997) 1462–1468.

[4] B.T. Kren, P. Bandyopadhyay, C.J. Steer, In vivo site-directed mutagenesis of the factor IX gene by chimeric RNA/DNA oligonucleotides, Nat. Med. 4 (1998) 285–290.

[5] B.T. Kren, B. Parashar, P. Bandyopadhyay, N. Roy Chowdhury, J. Roy Chowdhury, C.J. Steer, Correction of the UDP-glucuronosyltransferase gene defect in the Gunn rat model of Crigler–Najjar syndrome type I with a chimeric oligonucleotide, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 10349–10354.

[6] V. Alexeev, O. Igoucheva, A. Domashenko, G. Cotsarelis, K. Yoon, Localized in vivo genotypic and phenotypic correction of the albino mutation in skin by RNA–DNA oligonucleotide, Nat. Biotechnol. 18 (2000) 43–47.

[7] T.A. Rando, M.-H. Disatnik, L.Z.-H. Zhou, Rescue of dystrophin expression in *mdx* mouse muscle by RNA/DNA oligonucleotides, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 5363–6368.

[8] R.J. Bartlett, S. Stockinger, M.M. Denis, W.T. Bartlett, L. Inverardi, T.T. Le, N.T. Man, G.E. Morris, D.J. Bogan, J. Metcalf-Bogan, J.N. Kornegay, In vivo targeted repair of a point mutation in the canine dystrophin gene by a chimeric RNA/DNA oligonucleotide, Nat. Biotechnol. 18 (2000) 615–622.

[9] P. Bandyopadhyay, X. Ma, C. Linehan-Stieers, B.T. Kren, C.J. Steer, Nucleotide exchange in genomic DNA of rat hepatocytes using RNA/DNA oligonucleotides, J. Biol. Chem. 274 (1999) 10163–10172.

[10] P.R. Beetham, P.B. Kipp, X.L. Sawycky, C.J. Arntzen, G.D. May, A tool for functional plant genomics: chimeric RNA/DNA oligonucleotides cause in vivo gene-specific mutations, Proc. Natl. Acad. Sci. U. S. A. 96 1999, pp. 8774–8778.

[11] T. Zhu, D.J. Peterson, L. Tagliani, G. St. Clair, C.L. Baszczynski, B. Bowen, Targeted manipulation of maize genes in vivo using chimeric RNA/DNA oligonucleotides, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 8768–8773.

[12] T. Zhu, K. Mettenburg, D.J. Peterson, L. Tagliani, C.L. Baszczynski, Engineering herbicide-resistant maize using chimeric RNA/DNA oligonucleotides, Nat. Biotechnol. 18 (2000) 555–558.

[13] Z. Zhang, M. Eriksson, G. Falk, C. Graff, S.C. Presnell, M.S. Read, T.C. Nichols, M. Blombäck, M. Anvret, Failure to achieve gene conversion with chimeric circular oligonucleotides: potentially misleading PCR artifacts observed, Antisense Nucleic Acid Drug Dev. 8 (1998) 531–536.

[14] A. Cole-Strauss, H. Gamper, W.K. Holloman, M. Muñoz, N. Cheng, E.B. Kmiec, Targeted gene repair directed by the chimeric RNA/DNA oligonucleotide in a mammalian cell-free extract, Nucleic Acids Res. 27 (1999) 1323–1330.

[15] H.B. Gamper, A. Cole-Strauss, R. Metz, H. Parekh, R. Kumar, E.B. Kmiec, A plausible mechanism for gene correction by chimeric oligonucleotides, Biochemistry 39 (2000) 5808–5816.

[16] V.K. Jayasena, B.H. Johnston, Complement-stabilised D-loop: RecA-catalyzed stable pairing of linear DNA molecules at internal sites, J. Mol. Biol. 230 (1993) 1015–1024.

[17] H.B. Gamper, H. Parekh, M.C. Rice, M. Bruner, H. Youkey, E.B. Kmiec, The DNA strand of chimeric RNA/DNA oligonucleotides can direct gene repair/conversion activity in mammalian and plant cell-free extracts, Nucleic Acids Res. 28 (2000) 4332–4339.

[18] O. Igoucheva, V. Alexeev, K. Yoon, Targeted correction by small single-stranded oligonucleotides in mammalian cells, Gene Ther. 8 (2001) 391–399.

[19] G. van der Steege, P.H.L. Schuilenga-Hut, C.H.C.M. Buys, H. Scheffer, H.H. Pas, M.F. Jonkman, Persistent failures in gene repair, Nat. Biotechnol. 19 (2001) 305–306.

[20] A.D. Tagalakis, I.R. Graham, D.R. Riddell, J.G. Dickson, J.S. Owen, Gene correction of the apolipoprotein (apo) E2 phenotype to wild-type apoE3 by in situ chimeraplasty, J. Biol. Chem. 276 (2001) 13226–13230.

[21] I.R. Graham, A. Manzano, A.D. Tagalakis, G. Sperber, V. Hill, S. Beattie, S. Schepelmann, G. Dickson, J.S. Owen, Gene repair validation, Nat. Biotechnol. 19 (2001) 507–508.